

JC18 Rec'd PCT/PTO 2 5 MAY 2001

SEQUENCE LISTING

<110> Roche Diagnostics GmbH

<120> Thermostable enzyme promoting the fidelity of
thermostable DNA polymerases - for improvement of
nucleic acid synthesis and amplification in vitro

<130> 5304/OA/

<140>

<141>

<160> 21

<170> PatentIn Ver. 2.1

<210> 1

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 1

gaaacgagga tccatgctca aaatcgccac c

31

<210> 2

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 2

ttgttactg cagctacag tcaaacacag c

31

<210> 3

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 3

ggttatcgaa atcagccaca gcg

23

<210> 4

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 4

tggtacgtc tgaactggc acgtca

27

<210> 5

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 5

tggtacgtc tgaactggc acgtct

27

<210> 6

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 6

tggtacgtc tgaactggc acgtcc

27

<210> 7

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 7

tggtacgtc tgaactggc acgtat

27

<210> 8

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 8

agcttatcga tggcactttt cggggaaatg tgcg

34

<210> 9

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 9

agcttatcga taagcggatg cggggagcag acaagc

36

<210> 10

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 10

tggttgaatt catatatctt agagggaggg c

31

<210> 11

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 11

tgtgtctgca gaaaacatca aggggtcccat a

31

<210> 12

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 12

ggaagtacag ctcagagttc tgcagcaccc ctgc

34

<210> 13

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 13

caaagtcatg cggccatcgt tcagacacac c

31

<210> 14

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 14

ccttcactgt ctgcctaact ccttcgtgtg tccc

34

<210> 15

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 15

actgtgcttc ctgacccatg gcagaagcgc cttc

34

<210> 16

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 16

ccttctagag tcaactctag atgtggactt agag

34

<210> 17

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 17

gatgcgaaac tgaggctggc tgtactgtct c

31

<210> 18

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 18

gtcccaagca atggatgat

19

<210> 19

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 19

tggaacttt ccacttgat

19

<210> 20

<211> 774

<212> DNA

<213> *Archaeoglobus fulgidus*

<220>

<221> CDS

<222> (1)..(774)

<400> 20

atg ctc aaa atc gcc acc ttc aac gta aac tcc atc agg agc aga ctg	48
Met Leu Lys Ile Ala Thr Phe Asn Val Asn Ser Ile Arg Ser Arg Leu	
1 5 10 15	
cac atc gtg att ccg tgg ctg aag gag aac aag cct gac att cta tgc	96
His Ile Val Ile Pro Trp Leu Lys Glu Asn Lys Pro Asp Ile Leu Cys	
20 25 30	
atg cag gag acg aag gtt gag aac agg aag ttt cct gag gcc gat ttt	144
Met Gln Glu Thr Lys Val Glu Asn Arg Lys Phe Pro Glu Ala Asp Phe	
35 40 45	
cac cgc atc ggc tac cac gtc gtc ttc agc ggg agc aag gga agg aat	192
His Arg Ile Gly Tyr His Val Val Phe Ser Gly Ser Lys Gly Arg Asn	
50 55 60	
gga gtg gcc ata gct tcc ctc gaa gag cct gag gat gtc agc ttc ggt	240
Gly Val Ala Ile Ala Ser Leu Glu Glu Pro Glu Asp Val Ser Phe Gly	
65 70 75 80	
ctc gat tca gag ccg aag gac gag gac agg ctg ata agg gca aag ata	288
Leu Asp Ser Glu Pro Lys Asp Glu Asp Arg Leu Ile Arg Ala Lys Ile	
85 90 95	
gct ggc ata gac gtg att aac acc tac gtt cct cag gga ttc aaa att	336
Ala Gly Ile Asp Val Ile Asn Thr Tyr Val Pro Gln Gly Phe Lys Ile	
100 105 110	
gac agc gag aag tac cag tac aag ctc cag tgg ctt gag agg ctt tac	384
Asp Ser Glu Lys Tyr Gln Tyr Lys Leu Gln Trp Leu Glu Arg Leu Tyr	
115 120 125	
cat tac ctt caa aaa acc gtt gac ttc aga agc ttt gct gtt tgg tgt	432
His Tyr Leu Gln Lys Thr Val Asp Phe Arg Ser Phe Ala Val Trp Cys	
130 135 140	
gga gac atg aac gtt gct cct gag cca atc gac gtt cac tcc cca gac	480
Gly Asp Met Asn Val Ala Pro Glu Pro Ile Asp Val His Ser Pro Asp	
145 150 155 160	

aag ctg aag aac cac gtc tgc ttc cac gag gat gcg aga agg gca tac 528
 Lys Leu Lys Asn His Val Cys Phe His Glu Asp Ala Arg Arg Ala Tyr
 165 170 175

aaa aaa ata ctc gaa ctc ggc ttt gtt gac gtg ctg aga aaa ata cat 576
 Lys Lys Ile Leu Glu Leu Gly Phe Val Asp Val Leu Arg Lys Ile His
 180 185 190

ccc aac gag aga att tac acc ttc tac gac tac agg gtt aag gga gcc 624
 Pro Asn Glu Arg Ile Tyr Thr Phe Tyr Asp Tyr Arg Val Lys Gly Ala
 195 200 205

att gag cgg ggg ctg gga tgg agg gtt gat gcc atc ctc gcc acc cca 672
 Ile Glu Arg Gly Leu Gly Trp Arg Val Asp Ala Ile Leu Ala Thr Pro
 210 215 220

ccc ctc gcc gaa aga tgc gtg gac tgc tac gca gac atc aaa ccg agg 720
 Pro Leu Ala Glu Arg Cys Val Asp Cys Tyr Ala Asp Ile Lys Pro Arg
 225 230 235 240

ctg gca gaa aag cca tcc gac cac ctc cct ctc gtt gct gtg ttt gac 768
 Leu Ala Glu Lys Pro Ser Asp His Leu Pro Leu Val Ala Val Phe Asp
 245 250 255

gtg tag 774
 Val

<210> 21

<211> 258

<212> PRT

<213> *Archaeoglobus fulgidus*

<400> 21

Met Leu Lys Ile Ala Thr Phe Asn Val Asn Ser Ile Arg Ser Arg Leu
 1 5 10 15

His Ile Val Ile Pro Trp Leu Lys Glu Asn Lys Pro Asp Ile Leu Cys
 20 25 30

Met Gln Glu Thr Lys Val Glu Asn Arg Lys Phe Pro Glu Ala Asp Phe
 35 40 45

His Arg Ile Gly Tyr His Val Val Phe Ser Gly Ser Lys Gly Arg Asn
 50 55 60

Gly Val Ala Ile Ala Ser Leu Glu Glu Pro Glu Asp Val Ser Phe Gly
 65 70 75 80

Leu Asp Ser Glu Pro Lys Asp Glu Asp Arg Leu Ile Arg Ala Lys Ile
 85 90 95

Ala Gly Ile Asp Val Ile Asn Thr Tyr Val Pro Gln Gly Phe Lys Ile
 100 105 110

Asp Ser Glu Lys Tyr Gln Tyr Lys Leu Gln Trp Leu Glu Arg Leu Tyr
 115 120 125

His Tyr Leu Gln Lys Thr Val Asp Phe Arg Ser Phe Ala Val Trp Cys
130 135 140

Gly Asp Met Asn Val Ala Pro Glu Pro Ile Asp Val His Ser Pro Asp
145 150 155 160

Lys Leu Lys Asn His Val Cys Phe His Glu Asp Ala Arg Arg Ala Tyr
165 170 175

Lys Lys Ile Leu Glu Leu Gly Phe Val Asp Val Leu Arg Lys Ile His
180 185 190

Pro Asn Glu Arg Ile Tyr Thr Phe Tyr Asp Tyr Arg Val Lys Gly Ala
195 200 205

Ile Glu Arg Gly Leu Gly Trp Arg Val Asp Ala Ile Leu Ala Thr Pro
210 215 220

Pro Leu Ala Glu Arg Cys Val Asp Cys Tyr Ala Asp Ile Lys Pro Arg
225 230 235 240

Leu Ala Glu Lys Pro Ser Asp His Leu Pro Leu Val Ala Val Phe Asp
245 250 255

Val

5304us.ST25.txt
SEQUENCE LISTING

<110> Ankenbauer, Waltraud
Laue, Frank
Sobek, Harald
Greif, Michael

<120> THERMOSTABLE ENZYME PROMOTING THE FIDELITY OF THERMOSTABLE DNA
POLYMERASES - FOR IMPROVEMENT OF NUCLEIC ACID SYNTHESIS AND
AMPLIFICATION IN VITRO

<130> 5304

<150> PCT/EP00/09423

<151> 2000-09-27

<150> EP/99119268.3

<151> 1999-09-28

<160> 21

<170> PatentIn Ver. 3.0

<210> 1

<211> 31

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 1

gaaacgagga tccatgctca aaatcgccac c

31

<210> 2

<211> 31

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 2

ttgttcactg cagctacacg tcaaacacag c

31

5304us.ST25.txt

<210> 3
<211> 23
<212> DNA
<213> Artificial

<220>
<223> amplification primer

<400> 3
ggttatcgaa.atcagccaca gcg

23

<210> 4
<211> 27
<212> DNA
<213> Artificial

<220>
<223> amplification primer

<400> 4
tggatacgtc tgaactggtc acggtca

27

<210> 5
<211> 27
<212> DNA
<213> Artificial

<220>
<223> amplification primer

<400> 5
tggatacgtc tgaactggtc acggtct

27

<210> 6
<211> 27
<212> DNA
<213> Artificial

<220>
<223> amplification primer

<400> 6
tggatacgtc tgaactggtc acggtcc 27

<210> 7
<211> 27
<212> DNA
<213> Artificial

<220>
<223> amplification primer

<400> 7
tggatacgtc tgaactggtc acggtat 27

<210> 8
<211> 34
<212> DNA
<213> Artificial

<220>
<223> amplification primer

<400> 8
agcttatcga tggcactttt cggggaaatg tgcg 34

<210> 9
<211> 36
<212> DNA
<213> Artificial

<220>
<223> amplification primer

<400> 9
agcttatcga taagcggatg ccgggagcag acaagc 36

<210> 10
<211> 31
<212> DNA
<213> Artificial

<220>

<223> amplification primer

<400> 10

tggttgaatt catatatctt agagggaggg c

31

<210> 11

<211> 31

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 11

tgtgtctgca gaaaacatca agggccccat a

31

<210> 12

<211> 34

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 12

ggaagtacag ctcagagttc tgcagcaccc ctgc

34

<210> 13

<211> 31

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 13

caaagtcacg cggccatcgt tcagacacac c

31

<210> 14

<211> 34

5304us.ST25.txt

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 14

ccttcactgt ctgcctaact ccttcgtgtg tccc

34

<210> 15

<211> 34

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 15

actgtgcttc ctgacccatg gcagaagcgc cttc

34

<210> 16

<211> 34

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 16

ccttctagag tcaactctag atgtggactt agag

34

<210> 17

<211> 31

<212> DNA

<213> Artificial

<220>

<223> amplification primer

<400> 17

gatgcgaaac tgaggctggc tgtactgtct c

31

5304us.ST25.txt

<210> 18
 <211> 19
 <212> DNA
 <213> Artificial

<220>
 <223> amplification primer

<400> 18
 gtcccaagca atggatgat

19

<210> 19
 <211> 19
 <212> DNA
 <213> Artificial
 <220>
 <223> amplification primer

<400> 19
 tggaaacttt ccacttgat

19

<210> 20
 <211> 774
 <212> DNA
 <213> *Archaeoglobus fulgidus*

<220>
 <221> CDS
 <222> (1)..(774)

<400> 20
 atg ctc aaa atc gcc acc ttc aac gta aac tcc atc agg agc aga ctg 48
 Met Leu Lys Ile Ala Thr Phe Asn Val Asn Ser Ile Arg Ser Arg Leu
 1 5 10 15

cac atc gtg att ccg tgg ctg aag gag aac aag cct gac att cta tgc 96
 His Ile Val Ile Pro Trp Leu Lys Glu Asn Lys Pro Asp Ile Leu Cys
 20 25 30

atg cag gag acg aag gtt gag aac agg aag ttt cct gag gcc gat ttt 144
 Met Gln Glu Thr Lys Val Glu Asn Arg Lys Phe Pro Glu Ala Asp Phe
 35 40 45

5304us.ST25.txt

cac cgc atc ggc tac cac gtc gtc ttc agc ggg agc aag gga agg aat	192
His Arg Ile Gly Tyr His Val Val Phe Ser Gly Ser Lys Gly Arg Asn	
50 55 60	
gga gtg gcc ata gct tcc ctc gaa gag cct gag gat gtc agc ttc ggt	240
Gly Val Ala Ile Ala Ser Leu Glu Glu Pro Glu Asp Val Ser Phe Gly	
65 70 75 80	
ctc gat tca gag ccg aag gac gag gac agg ctg ata agg gca aag ata	288
Leu Asp Ser Glu Pro Lys Asp Glu Asp Arg Leu Ile Arg Ala Lys Ile	
85 90 95	
gct ggc ata gac gtg att aac acc tac gtt cct cag gga ttc aaa att	336
Ala Gly Ile Asp Val Ile Asn Thr Tyr Val Pro Gln Gly Phe Lys Ile	
100 105 110	
gac agc gag aag tac cag tac aag ctc cag tgg ctt gag agg ctt tac	384
Asp Ser Glu Lys Tyr Gln Tyr Lys Leu Gln Trp Leu Glu Arg Leu Tyr	
115 120 125	
cat tac ctt caa aaa acc gtt gac ttc aga agc ttt gct gtt tgg tgt	432
His Tyr Leu Gln Lys Thr Val Asp Phe Arg Ser Phe Ala Val Trp Cys	
130 135 140	
gga gac atg aac gtt gct cct gag cca atc gac gtt cac tcc cca gac	480
Gly Asp Met Asn Val Ala Pro Glu Pro Ile Asp Val His Ser Pro Asp	
145 150 155 160	
aag ctg aag aac cac gtc tgc ttc cac gag gat gcg aga agg gca tac	528
Lys Leu Lys Asn His Val Cys Phe His Glu Asp Ala Arg Arg Ala Tyr	
165 170 175	
aaa aaa ata ctc gaa ctc ggc ttt gtt gac gtg ctg aga aaa ata cat	576
Lys Lys Ile Leu Glu Leu Gly Phe Val Asp Val Leu Arg Lys Ile His	
180 185 190	
ccc aac gag aga att tac acc ttc tac gac tac agg gtt aag gga gcc	624
Pro Asn Glu Arg Ile Tyr Thr Phe Tyr Asp Tyr Arg Val Lys Gly Ala	
195 200 205	
att gag cgg ggg ctg gga tgg agg gtt gat gcc atc ctc gcc acc cca	672
Ile Glu Arg Gly Leu Gly Trp Arg Val Asp Ala Ile Leu Ala Thr Pro	
210 215 220	

5304us.ST25.txt

ccc ctc gcc gaa aga tgc gtg gac tgc tac gca gac atc aaa ccg agg 720
 Pro Leu Ala Glu Arg Cys Val Asp Cys Tyr Ala Asp Ile Lys Pro Arg
 225 230 235 240

ctg gca gaa aag cca tcc gac cac ctc cct ctc gtt gct gtg ttt gac 768
 Leu Ala Glu Lys Pro Ser Asp His Leu Pro Leu Val Ala Val Phe Asp
 245 250 255

gtg tag 774
 Val

<210> 21

<211> 258

<212> PRT

<213> Archaeoglobus fulgidus

<400> 21

Met Leu Lys Ile Ala Thr Phe Asn Val Asn Ser Ile Arg Ser Arg Leu
 1 5 10 15

His Ile Val Ile Pro Trp Leu Lys Glu Asn Lys Pro Asp Ile Leu Cys
 20 25 30

Met Gln Glu Thr Lys Val Glu Asn Arg Lys Phe Pro Glu Ala Asp Phe
 35 40 45

His Arg Ile Gly Tyr His Val Val Phe Ser Gly Ser Lys Gly Arg Asn
 50 55 60

Gly Val Ala Ile Ala Ser Leu Glu Glu Pro Glu Asp Val Ser Phe Gly
 65 70 75 80

Leu Asp Ser Glu Pro Lys Asp Glu Asp Arg Leu Ile Arg Ala Lys Ile
 85 90 95

Ala Gly Ile Asp Val Ile Asn Thr Tyr Val Pro Gln Gly Phe Lys Ile
 100 105 110

Asp Ser Glu Lys Tyr Gln Tyr Lys Leu Gln Trp Leu Glu Arg Leu Tyr
 115 120 125

His Tyr Leu Gln Lys Thr Val Asp Phe Arg Ser Phe Ala Val Trp Cys
 130 135 140

5304us.ST25.txt

Gly Asp Met Asn Val Ala Pro Glu Pro Ile Asp Val His Ser Pro Asp
145 150 155 160

Lys Leu Lys Asn His Val Cys Phe His Glu Asp Ala Arg Arg Ala Tyr
165 170 175

Lys Lys Ile Leu Glu Leu Gly Phe Val Asp Val Leu Arg Lys Ile His
180 185 190

Pro Asn Glu Arg Ile Tyr Thr Phe Tyr Asp Tyr Arg Val Lys Gly Ala
195 200 205

Ile Glu Arg Gly Leu Gly Trp Arg Val Asp Ala Ile Leu Ala Thr Pro
210 215 220

Pro Leu Ala Glu Arg Cys Val Asp Cys Tyr Ala Asp Ile Lys Pro Arg
225 230 235 240

Leu Ala Glu Lys Pro Ser Asp His Leu Pro Leu Val Ala Val Phe Asp
245 250 255

Val